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PRINCIPAL INVESTIGATOR: Dan Liebermann, Ph.D.

CONTRACTING ORGANIZATION: Temple University School of Medicine
Philadelphia, PA 19140

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Dan Liebermann, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Temple University School of Medicine
Philadelphia, PA 19140

E-Mail: lieberma@temple.edu

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The overall theme of the proposed research is to examine whether deficiency in the Growth.Arrest & DNA Damage (Gadd)45 family of genes (Gadd45a & Gadd45b), playing important roles in controlling cell division and response of cells to anti-cancer agents, contributes to breast carcinogenesis. To this end, a multifaceted research plan takes advantage of established models of breast cancer prone mice (Balb/c, MMTV-ras, MMTV-c-myc) that are being crossed with mouse strains deficient for Gadd45a or Gadd45a/b to generate breast cancer prone mouse strains deficient for Gadd45 genes. Such mouse strains will provide attractive animal models to assess how deficiency in Gadd45 genes promotes breast carcinogenesis under different experimental settings (i.e. with or without hormone stimulation, irradiation or the chemical carcinogen DMBA). We have successfully accomplished the generation of mouse strains that are deficient in the Gadd4a gene and are prone to breast cancer due to oncogenic myc (MMTV-c-myc Gadd45a-/-) or ras (MMTV-c- Gadd45a-/-). Preliminary observations suggest that haplo-deficiency of Gadd45a accelerates myc or ras driven breast carcinogenesis, predicting even more pronounced effects in MMTV-v-ras and MMTV-c-myc mice that are null for Gadd45a or null for both Gad45a & b genes

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INTRODUCTION:

The development of breast cancer is a multistage process. Alterations in multiple genes, that control cell proliferation, survival and metastasis, are known to co-operate in breast tumorigenesis. The overall theme of the proposed research is to examine whether deficiency in the so called Growth.Arrest & DNA Damage Gadd45 family of genes (Gadd45a & Gadd45b), playing important roles in controlling cell division and response of cells to anti-cancer agents, contributes to breast carcinogenesis. Towards this end, a multifaceted research plan is taking advantage of established models of breast cancer prone mice that are crossed with mouse strains deficient for Gadd45a or Gadd45a/b to examine how deficiency in Gadd45 genes may promote breast carcinogenesis. Explicitly, to assess the effect of Gadd45a & Gadd45a/b deficiencies on breast tumorigenesis promoted by hormone, ionizing radiation (IR) or chemical carcinogen the Gadd45a-/- and Gadd45a/b-/- alleles are being transferred onto the cancer prone Balb/c genetic background (Task I). Expanding on this research, in Task II, Gadd45a-/- and Gadd45a/b-/- mice are crossed with c-myc and v-ras transgenic mice to assess the effect of Gadd45a & Gadd45 a/b deficiencies on breast carcinogenesis promoted by oncogenic ras or myc.

BODY

Task I: Assess the effects of Gadd45 deficiencies on breast cancer tumorigenesis induced by hormone, IR or chemical carcinogen using breast cancer susceptible Balb/c Med (i.e. Balb/c) mice.

A. Transfer Gadd45a-/- and Gadd45 a/b-/- null alleles onto the genetic background of breast cancer prone Balb/cMed mice, by backcrossing for 5 generations. (**Months 1-10**)

B. Assess the effect of Gadd45a & Gad45a/b deficiencies on breast tumorigenesis in Balb/c mice following hormone stimulation, treatment with IR or DMBA (**Months 10-24**)

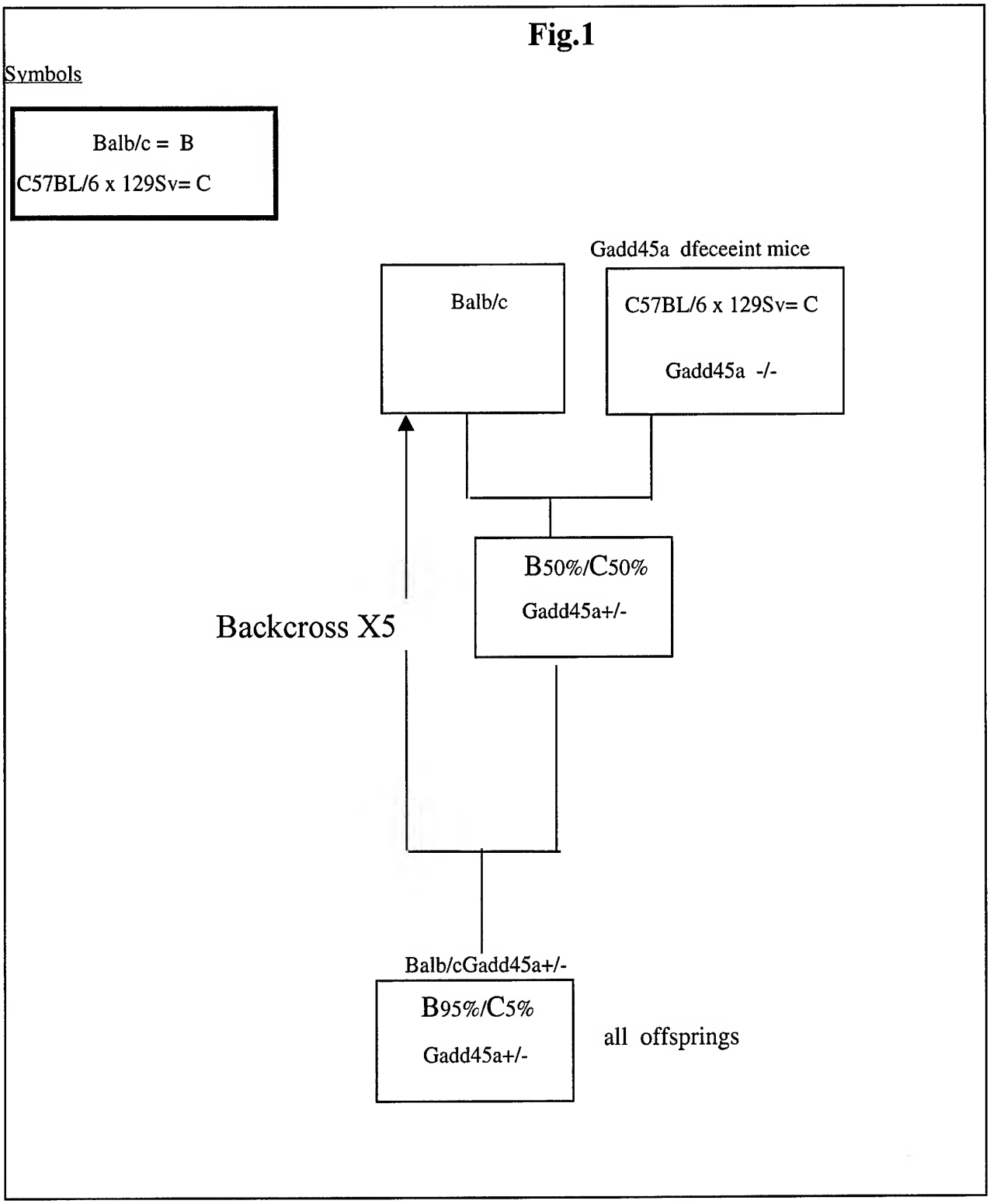
C. Cellular/molecular characterization of breast tumors promoted by Gadd45 deficiency & hormone stimulation, or treatment with IR or DMBA (**Months 20-36**)

A. Transfer the Gadd45a-/- and Gadd45 a/b-/- null alleles onto the genetic background of breast cancer prone Balb/cMed mice, by backcrossing for 5 generations. (**Months 1-10**)

We are in the laborious process of transferring Gadd45α -/- and Gadd45α/b -/- alleles from the mixed C57BL/6 x 129Sv background onto the genetic background of the breast cancer prone Balb/c strain of mice (1), by backcrossing for five generations..

We are completing the 5th and final round of backcrossing of the Gadd45α -/- allele onto the Balb/c genetic background to obtain offsprings which essentially have the Balb/c genetic background and are heterozygous for Gadd45a deficiency (Balb/cGadd45a+/-) **(Fig.1).**

Fig.1



Following completion of the 5th backcrossing Balb/cGadd45a+/- progeny mice will be mated to obtain Balb/c mice that are deficient for Gadd45a (Balb/cGadd45a-/- mice). These mice will be used to generate 16 Balb/cGadd45a-/- mice per treatment group to continue with Task1B (see below)

1.Balb/c WT mice

- untreated	16
-multiparous	16
-DMBA	16
-IR	16

2. Balb/c Gadd45a-/- mice

-untreated	16
-multiparous	16
-DMBA	16
-IR	16

On the other hand, the number of Gadd45a/b-/- double knockout mice available for experiments has been, so far , limited. Consequently backcrossing of Gadd45a/b-/- mice onto the Balb/c background was slowed down, and we are at the 3rd round of backcrossing (using the mating scheme depicted in **Fig. 1**).

Task II: Assess the effects of Gadd45 deficiencies on oncogene driven breast carcinogenesis.

A. Establishment of MMTV-v-ras (i.e. MMTV-ras) and WAP-c-myc (or MMTV-c-myc) mice that are deficient for Gadd45a or Gadd45a/b **(Months 1-14)**

B. Effect of Gadd45a & Gadd45a/b deficiencies on breast cancer development in MMTV-v-ras and WAP-c-myc (or MMTV-c-myc) mice that are WT or deficient for Gadd45a or Gadd45a/b expression will be explored following the same path as described in AIM 1B **(Months 14-20).**

C. Cellular/molecular characterization of breast tumors promoted by Gadd45 deficiency and oncogenic ras or c-myc will be ascertained following the same path as described in AIM 1C. **(Months 20-36)**

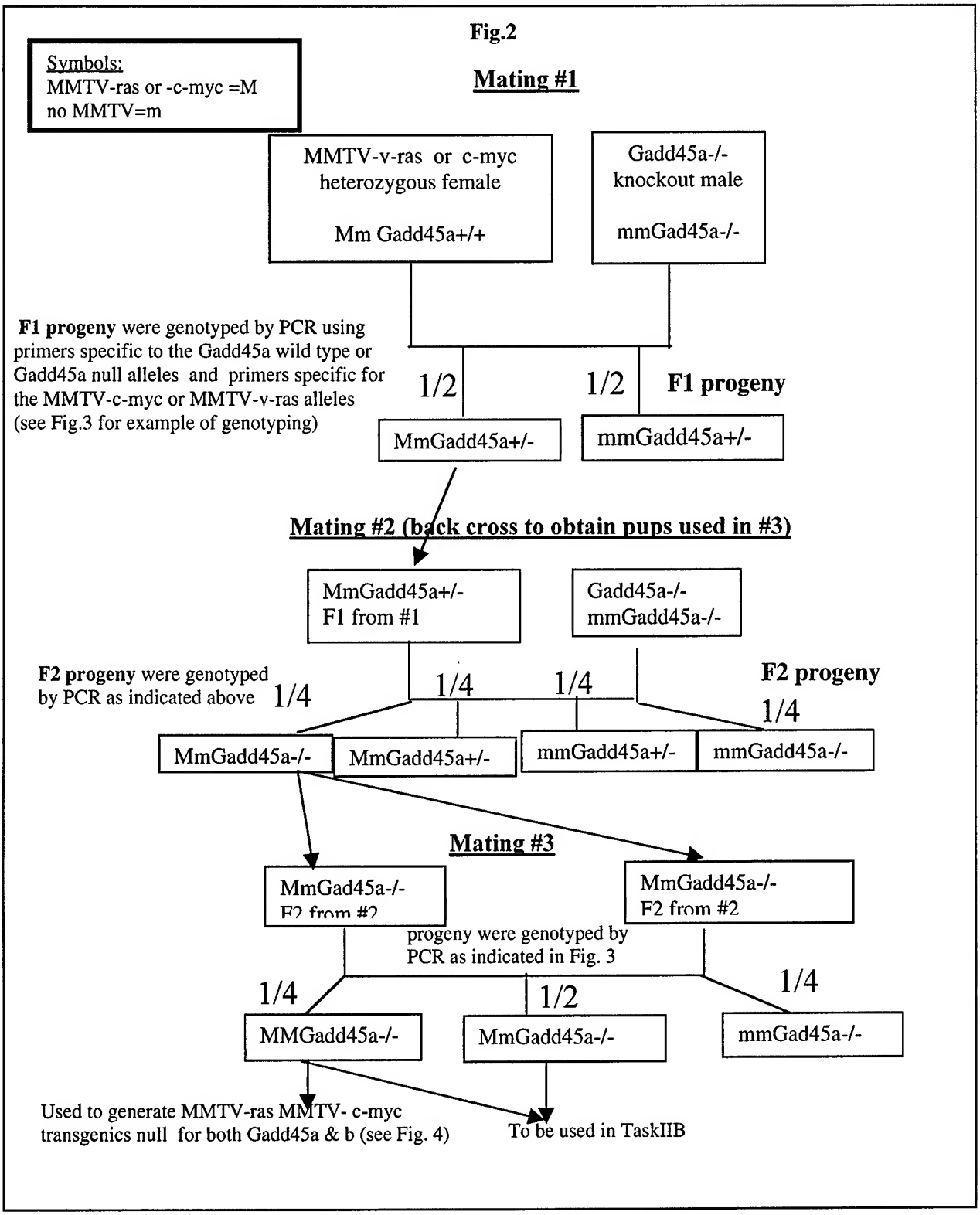
MMTV-c-myc and the WAP-c-myc mice are two documented mouse models that have been used extensively to study c-myc driven breast carcinogenicity (2). Originally we have opted to use WAP-c-myc mice, since the WAP promoter is known to be more breast cell specific compared to the MMTV promoter (3) . However, upon initiating the work (and along reviewers comments) it became apparent that using MMTV-c-myc mice instead of WAP-c-myc mice may provide a more attractive experimental model to assess the effect of Gadd45 deficiency on oncogene (i.e deregulated c-myc , activated ras) driven breast carcinogenes:

1) In both of these oncognic mouse models the same promoter, i.e. the MMTV promoter, drives expression of oncogenic myc and activated ras with similar patterns and level of expression in the mammary tissue (4-5). Thus, co-operating effects of Gadd45 deficiency on myc or ras driven mammary tumor development are more directly comparable. For example, MMTV-ras and MMTV-c- myc transgenic mice recently have been used to compare the effects of the cyclin dependent kinase inhibitor p21 deficiency on c-myc or ras driven breast carcinogenicity (5). It is noteworthy, that p21, similar to Gadd45a,, is a p53 target gene that mediates p53 dependent growth arrest.

2) MMTV v-ras mice develop spontaneous mammary adenocarcinomas starting at 3-6 months of age (4-5), whereas MMTV-c-myc mice develop similar tumors at 6-9 months of age (4-5). On the other hand , 80% of multiparous WAP-c-myc female mice develop multiple mammary tumors as early as 2 months (3). Thus, it is clear that co-operating effects of Gadd45 deficiency on c-myc driven breast carcinogenesis, anticipated to accelerate the timing and incidence of tumor formation, will be more pronounced and easier to study in MMTV-c-myc mice rather than in WAP-c-myc mice.

Thus, MMTV-v-ras and MMTV-c-myc transgenic mice (heterozygous for the MMTV-v-ras or MMTV-c-myc alleles), were crossed with Gadd45a-/- mice, to generate MMTV-ras Gadd45α-/- mice and MMTV-myc Gadd45α-/- mice , employing the mating scheme depicted in **Fig. 2**.

Fig.2



Litters (**Fig 2 matings #2 & #3**) were genotyped by PCR, using DNA from tail clips, to screen for pups that have the desired MMTV-ras Gadd45α-/- or MMTV-myc Gadd45α-/- genotype (**Fig. 3**). Currently we already have 4 MMTV-ras Gadd45α-/- mice and 2 MMTV-myc Gadd45α-/- mice that will be used to generate 12 mice per treatment group to continue with Task 2B

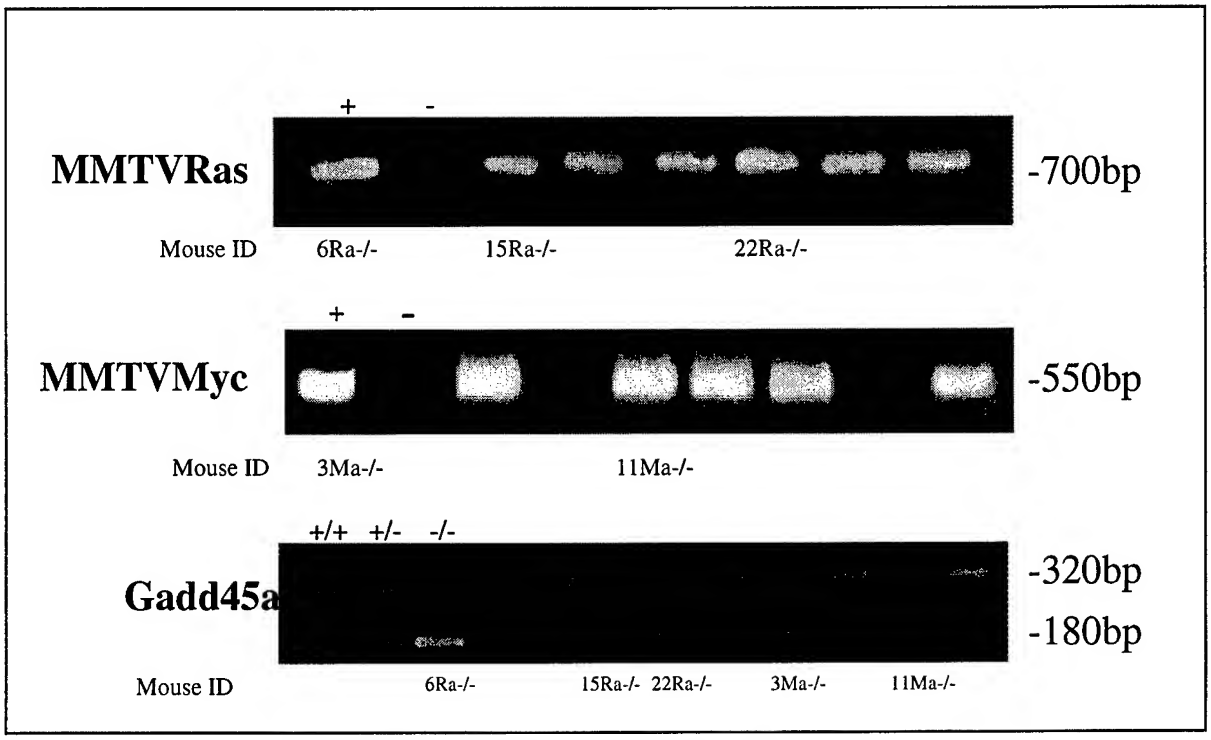


Fig. 3 PCR Genotyping for MMTV-Ras, MMTV-Myc and Gadd45α

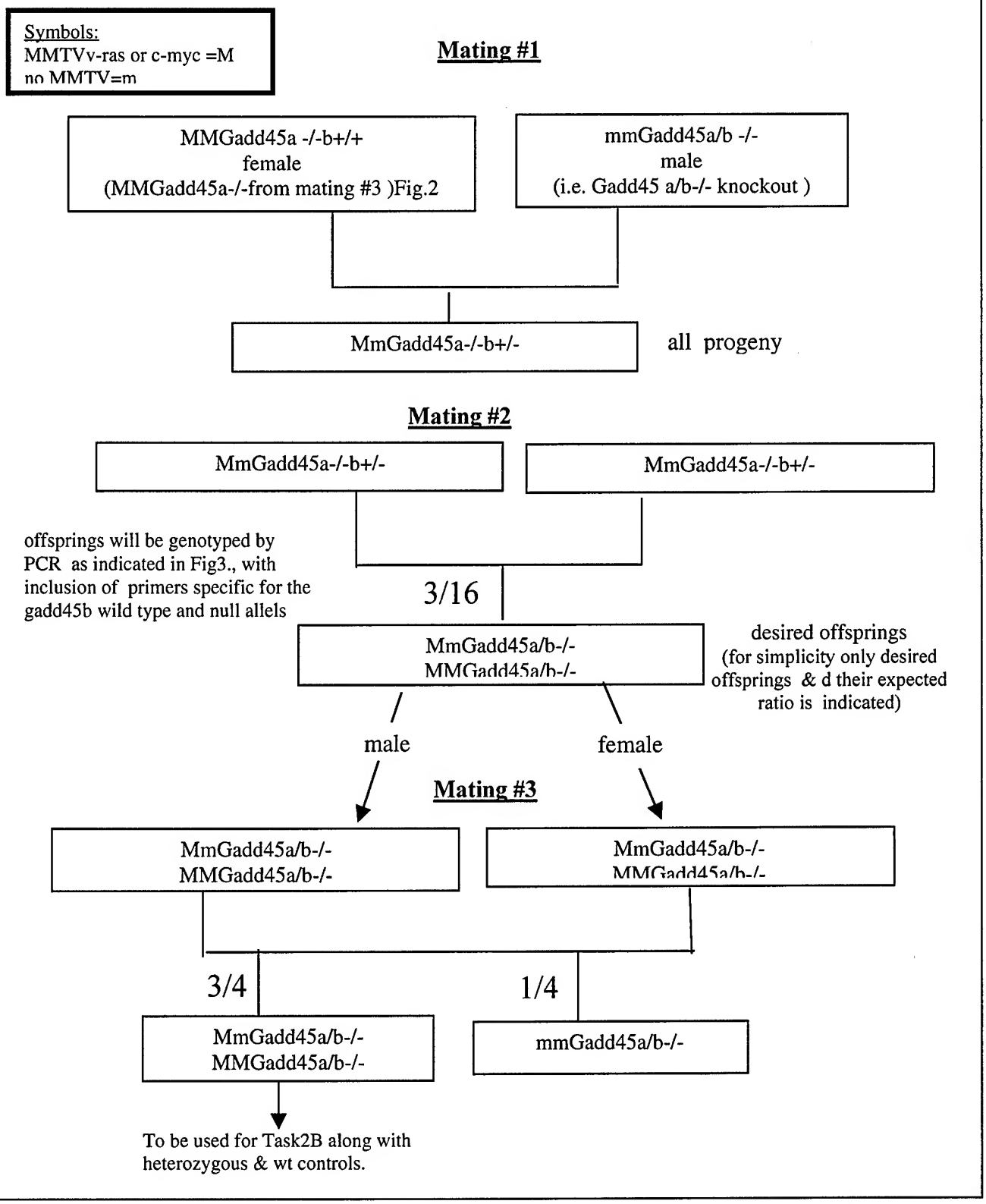
Genomic DNA was extracted from 4 week old mouse tail clips, using standard protocols. PCRs were performed using primers specific for MMTV-Ras, MMTV-myc and Gadd45α. Expected band sizes are as follows: Ras-700bp, Myc-550, Gadd45α WT-320, Neo-180. Mice which are MMTV-rasGadd45a-/- (6Ra-/-, 15a-/-, 22a-/-) or MMTV-c-mycGadd45a-/- (3Ma-/-, 11Ma-/-) are indicated.

Interestingly, although the incidence of tumor formation in mice used for breedings has not been yet rigorously quantitated, it appears that the incidence of tumor formation in MMTV-ras Gadd45α+/- or MMTV-myc Gadd45α+/- mice, that are haplo- deficient for the Gadd45a gene, is several fold (3-4 fold) higher than what was observed in MMTV-ras or MMTV-c-myc mice. Furthermore, several MMTV--ras Gadd45α+/- mice (4 mice) and MMTV-myc Gadd45α+/- mice (3 mice) developed multiple mammary and/or salivary gland tumors, whereas no multiple tumors were observed in MMTV-ras or MMTV-c-myc mice, within the same period of time (4-9 months). These preliminary observations strongly suggest that Gadd45a haplo-deficiency by itself is sufficient to accelerate mammary tumor development which is promoted by oncogenic myc or ras. These findings also predict that a greater effect might be observed in MMTV-ras and MMTV-c-myc mice that are null for the Gadd45a gene.

Careful analysis of the effect of Gadd45a status on breast cancer development in MMTV-ras and MMTV-c-myc mice that are either wild type, haplo- deficient or null for the Gadd45a gene, as delineated in Task2B, should be instrumental in determining how and under what experimental settings (i.e. with or without hormone stimulation, irradiation or DMBA) Gadd45a deficiency co-operates with oncogenic myc and ras in mammary tumorigenesis.

Mice that are homozygous for the MMTV-c-myc or the MMTV-v-ras alleles and are null for Gadd45a (i.e. MMGadd45a-/-; see Fig.2 mating #3) will be used to generate MMTV-ras Gadd45a/b-/- and MMTV-myc Gadd45a/b-/- mice as delineated in **Fig. 4.**

Fig. 4



KEY RESEARCH ACCOMPLISHMENTS:

* Generation of MMTV-ras Gadd45α-/- mice.
* Generation of MMTV-myc Gadd45α-/- mice.

REPORTABLE OUTCOMES:

MMTV-ras Gadd45α-/- mice and MMTV-myc Gadd45α-/- mice provide attractive models to study the effect of Gadd45 deficiencies on oncogene driven breast carcinogenesis. These mouse models can be further exploited, by crossing with other mouse strains of interest, to explore how compounded alterations in the expression of tumor suppressor genes and oncogenes co-operate in breast carcinognesis .

CONCLUSIONS:.

Summary of results. Task I. We are at the 5th round of backcrossing the Gadd45α -/- alleles onto the breast cancer prone Balb/c genetic background and at the 3rd round of backcrossing the Gadd45a/b -/- alleles onto the Balb/c background. This should enable us within the second year of the granting period to start and assess the effect of Gadd45a & Gad45a/b deficiencies on breast tumorigenesis in Balb/c mice as delineated in TaskIB.
TaskII. We have successfully accomplished the generation of mouse models (i.e. MMTV-v-ras Gadd45a-/- and MMTV-c-myc Gadd45a-/- mice) that will enable us within the second year of the granting period, as delineated in TaskIIB, to determine the effects of Gadd45a deficiency on breast carcinogensis driven by oncogenic myc or ras. Generation of the mouse strains that are oncogenic for myc or ras and are null for both Gadd45a &b is in progress. Preliminary observations suggest that already haplo -deficiency of Gadd45a accelerates myc or ras driven breast carcinogenesis, predicting an even more pronounced effect in MMTV-v-ras and MMTV-c-myc mice which are null for Gadd45a or null for both the Gadd45a & b genes.

Recommend changes on future work. Upon completion of the first year of the granting period we realize that the current research scheme is overly ambitious (admittingly as indicated by the reviewers), and needs focusing to obtain in depth and meaningful results that can be documented at the end of the granting period. The work which involves breeding , and keeping track of multiple mouse strains is immense and time consuming and the cost of housing the numerous mouse strains that are being generated is escalating. This large body of work will most likely expand exponentially in the second year of the granting period, once tumors are obtained, analyzed and tumor cell lines established.

Task I and Task II overlap in most, if not all, aspects of the work and share the same overall objective. Essentially they provide a sort of redundancy to increase the chance of success. Given the research dynamics, Task II has advanced much more rapidly, and already yielded interesting results which increase its likelihood of success. Thus, we believe that focusing on Task II and pursuing it more aggressively in years 2 & 3 of the funding periods will enable us to better address the overall objective of the research, and consequently increase the significance and quality of reportable outcomes.

Over all significance of the research ("so what section"). The overall objective of the research scheme is to generate mouse models that will enable us to assess how deficient expression of the growth suppressive and stress induced Gadd45 family of genes, which are transcriptional targets for p53, BRCA1, & TGFb, contribute to breast tumorigenesis under diverse experimental settings . Results obtained should provide information that will increase understanding of the molecular basis for breast cancer development, and may be utilized to design rational, novel therapies for treatment of breast cancer patients.

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